UNIVERSITY OF UTAH COLLEGE OF PHARMACY

FINAL READING APPROVAL

TO THE DOCTOR OF PHARMACY COMMITTEE OF THE UNIVERSITY OF UTAH COLLEGE OF PHARMACY:

I have read the clinical research project report of Dawn Callison Ford in its final form and have found that 1) its format, citations, and bibliographic style are consistent and acceptable; 2) its illustrative materials including figures, tables, and charts are in place; and 3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Doctor of Pharmacy Committee.

6-13-83
Date

Chairman, Supervisory Committee

Approved for the Department of Pharmacy Practice

Chairman

Approved for the Doctor of Pharmacy Committee

Chairman, Doctor of Pharmacy Committee

UNIVERSITY OF UTAH COLLEGE OF PHARMACY

SUPERVISORY COMMITTEE APPROVAL

of a clinical research project report submitted by

Dawn Callison Ford

We, the undersigned, have read this clinical research project report and have found it to be of satisfactory quality for a Doctor of Pharmacy Degree.

6-13-83
Date

Chairman, Supervisory Committee

6-8-83
Date

Member, Supervisory Committee

11 June 1983
Date

Member, Supervisory Committee

A COMPARISON OF HIGH PRESSURE LIQUID CHROMATOGRAPHY AND MICROBIOLOGICAL ASSAYS FOR CEFTAZIDIME

by

Dawn Callison Ford

A project submitted to the faculty of the
University of Utah in partial fulfillment of the requirements
for the degree of

Doctor of Pharmacy

College of Pharmacy

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INTRODUCTION

Ceftazidime (GR 20263) is an investigational cephalosporin antibiotic with a broad spectrum of activity. In early in vitro studies, antibacterial activity has been demonstrated against most aerobic gram-negative organisms, including Pseudomonas aeruginosa.[1-3] Harper found that it had mean minimum inhibitory concentrations (MICs) against Enterobacteriaceae of 0.07-0.5 mG/L with at least 90 percent of the isolates sensitive to 8 mG/L or less.[1] Phillips et al, compared ceftazidime with cefotaxime and moxalactam and demonstrated that ceftazidime had MICs similar to the other two antibiotics against Enterobacteriaceae, while its MICs against Pseudomonas aeruginosa were generally in the 1-4 mG/L range compared with cefotaxime and moxalactam MICs of 8-64 mG/L.[2] Like cefotaxime and moxalactam, ceftazidime has activity against most gram-positive organisms, but is less active than the penicillins and first-generation cephalosporins. Organisms usually resistant to ceftazidime include anaerobic bacteria, enterococci, methicillin-resistant staphylococci, and Listeria monocytogenes.[3] Because of its activity against aerobic gram-negative bacilli, beta-lactamase-positive and -negative Haemophilus influenzae, Streptococcus pneumoniae, and Neisseria meningitidis,[1] which are the primary bacterial pathogens of meningitis, the drug may have a role in the treatment of central nervous system infections. Sakata et al, found that ceftazidime penetrated into the cerebrospinal fluid of rabbits with experimentally-induced Streptococcus pneumoniae, Haemophilus influenzae

type b, or Escherichia coli meningitis and achieved cerebrospinal fluid bacteriocidal titers that ranged from 1:64 to 1:128.[4]

Before the pharmacokinetics of a new antibiotic such as ceftazidime can be adequately described, an accurate and reliable drug assay must be developed. Two methods currently available for ceftazidime determination include microbiological and high-pressure liquid chromatographic assays.[5,6]

There are several different microbiologic antibiotic assay techniques, including agar diffusion, turbimetric, inhibition of pH change, and radiometric. The one most commonly used is the agar diffusion technique.[8] With this method, nutrient agar plates are innoculated with a specific microbe and then the antimicrobial is placed on the plate in a paper disk or some type of reservoir. After an incubation period, zones of inhibition around the antimicrobial are measured. By comparing these zones with those produced by various standard solutions of the antibiotic, the concentration of the antibiotic can be determined. With good technique, this method can give sensitive and accurate results.[7] Advantages of this method include the need for minimal equipment, the low cost of necessary reagents,[8] the capacity to evaluate a large number of samples with minimal additional labor, and the fact that only substances with biological activity are measured.[6] Disadvantages include the long incubation time before results can be read, the ability of other antibiotics in the sample to interfere with results,[8] and the inability to detect metabolites with no biologic activity.[6]

High pressure liquid chromatography (HPLC) is a versatile chemical separation technique which has been used to separate and measure a wide

array of substances ranging from petrochemicals and pesticides to drugs.[9] With HPLC, a small volume of liquid containing the dissolved sample is injected into a moving liquid carrier. This mixture flows through the chromatographic column, which is packed with adsorbent particles. Due to chemical forces, the compounds in the sample adsorb onto the column in varying amounts, and an equilibrium is established between the column and carrier liquid. Adsorption onto the column increases the retention time, and will separate the compounds of interest. Eventually the compounds elute one at a time from the column. As they emerge, they flow through a sensitive detector which detects the compound and allows its concentration to be measured. The most commonly used detectors measure ultraviolet light absorptivity, refractive index, or electrical conductance. The detector sends electrical signals to a chart recorder, which plots the series of peaks representing the compounds eluting from the column.[9] Advantages of this method for measuring drug concentrations include high sensitivity and specificity,[8] the ability to differentiate between closely related compounds,[6,10] the ability to detect very small concentrations of the drug,[10] and the short amount of time needed to obtain results. Disadvantages include the high cost of the equipment, and the fact that the process requires much labor when samples cannot be prepared in advance and automatically injected into the apparatus (as is the case with unstable drugs).

The objective of this study was to compare the accuracy of an HPLC assay with a microbiological assay in the determination of serum ceftazidime concentrations.

MATERIALS AND METHODS

Study Design

An independent party added known concentrations of ceftazidime to samples of pooled human serum. These blinded samples were then assayed for concentrations of ceftazidime using an agar diffusion microbiologic assay and an HPLC assay performed in two separate laboratories. The results of the two assays were then compared with the known concentrations of the samples.

HPLC Assay

Apparatus. The HPLC system consisted of a gradient liquid chromatograph with a fixed wavelength (254 nm) ultraviolet light detector, model 332 (Beckman Instruments, Inc., Berkeley, CA), an autoinjector, WISP 710B (Waters Associates, Milford, MA), and a chart recorder, model 555 (Linear Instruments, Reno, NV). Chromatography was performed on a 100 mm x 4.6 mm stainless steel column packed with Hypersil ODS® (Allech Associates, Deerfield, IL).

Internal standard. The internal standard used was hydrochlorothiazide obtained from USP Reference Standards, Rockville, MD.

Sample Preparation. 100 µL of the serum sample were added to 200 µL methanol (containing the internal standard) in a conical centrifuge tube. This was vortexed for 20 seconds and allowed to stand at room temperature for one minute. It was then centrifuged at 2500-3000 rpm for 15 minutes, and the supernatant used for the assay. Because of the instability of the drug, samples were prepared within 30 minutes of injection onto the column.

Procedure. 15 µL of sample supernatant were injected onto the HPLC column and eluted at a flow rate of 2 mL/min. The mobile phase consisted of glacial acetic acid 10 mL, acetonitrile 25 mL, and deionized water q.s. ad 1000 mL. The pH was adjusted to 4 with 5N sodium hydroxide. A standard curve was prepared using samples containing known concentrations of ceftazidime. Peak heights of hydrochlorothiazide and ceftazidime were measured and the curve defined by the regression of peak height ratio versus ceftazidime concentration. Retention time relative to the solvent front was three minutes for the internal standard and five minutes for the ceftazidime.

Microbiological Assay

Method. Agar diffusion technique on Petri dishes was used.

Preparation of solutions. 100 mG ceftazidime powder and 10 mG anhydrous sodium carbonate were mixed with 35 mL distilled water. Varying amounts of this stock solution were then mixed with pooled human serum to make the standard solutions. The standard solutions ranged in concentration from 0.625 µG/mL to 30 µG/mL.

Preparation of assay plates. Oxoid (Bathingstokes, England) Antibiotic Medium #2 broth was prepared by adding 20.5 G broth to 1 L distilled water. One-half mL of overnight soy broth culture of Morganella morganii NTC #235 was added to 18 mL of the antibiotic medium broth and the mixture was poured into a 150 mM x 15 mM Petri

dish and allowed to solidify. Filter paper disks were impregnated with 20 μL each of ceftazidime solutions and placed on the plates. Each plate contained two unknown and four standard solutions of ceftazidime. All samples were run in duplicate. The plates were then incubated at 37°C on a flat, level surface for 4-6 hours and for 16-18 hours.

Determination of ceftazidime concentrations. The diameter of the zone of inhibition around each disk was measured with vernier calipers. Exponential regression analysis of zone diameter and corresponding concentration of standards was used to plot a regression line on semi-log graph paper. The concentrations of the unknown samples were then determined by reference to this standard curve.

Statistical Analysis

A student's t-test for paired data was used to compare the results of each assay with the known concentrations of ceftazidime.

RESULTS

HPLC Assay

The separation of the internal standard and the ceftazidime was complete and the chromatograph showed sharp, separate peaks (Figure 1). The standard curve was linear with a correlation coefficient of 0.92 (Figure 2).

The comparison of drug concentrations determined by the HPLC assay versus the known concentrations demonstrated no significant difference between the two sets of values ($p > 0.05$) (Table 1). The maximum percentage of difference between any single measurement and the true concentration was 12.6 percent. The mean percentage of difference for all the samples was 5.1 percent.

Microbiological Assay

Comparison of the drug concentrations determined by the microbiological assay versus the known concentrations demonstrated a significant difference between the two sets of values ($p < 0.05$) (Table 2). The maximum percentage of difference between any single measurement and the true concentration was 20.3 percent. The mean percentage of difference for all the samples was 10 percent. Results from one sample (#11) were not obtained due to technical difficulties. The correlation coefficient between the microbiological assay and the HPLC assay results was 0.985.

DISCUSSION

The purpose of this study was to compare the accuracy of an HPLC assay with a microbiological assay in determining serum ceftazidime concentrations. In contrast to other work which found no significant differences between an HPLC assay and an agar diffusion microbiological assay,[6] this study found the HPLC assay to be more accurate. The HPLC assay produced results that were not significantly different from the known concentrations of the samples, while the microbiological assay did show statistically significantly different results. The accuracy of the microbiological assay ranged from no to 20 percent error for any one sample. While this amount of error may be acceptable for routine clinical use, it could substantially alter results of pharmacokinetic studies. Ceftazidime is an unstable drug in solution and therefore, the HPLC assay is labor-intensive in this situation. That, along with the high initial cost of an HPLC apparatus, may make a microbiological

assay more appropriate for routine clinical practice. However, it is apparent that some degree of accuracy will be lost if a microbiological assay is used in preference to an HPLC assay.

FIGURES

#1–Solvent Front
#2–Hydrochlorothiazide
#3–Ceftazidime

Figure 1. EXAMPLE CHROMATOGRAPH PEAK

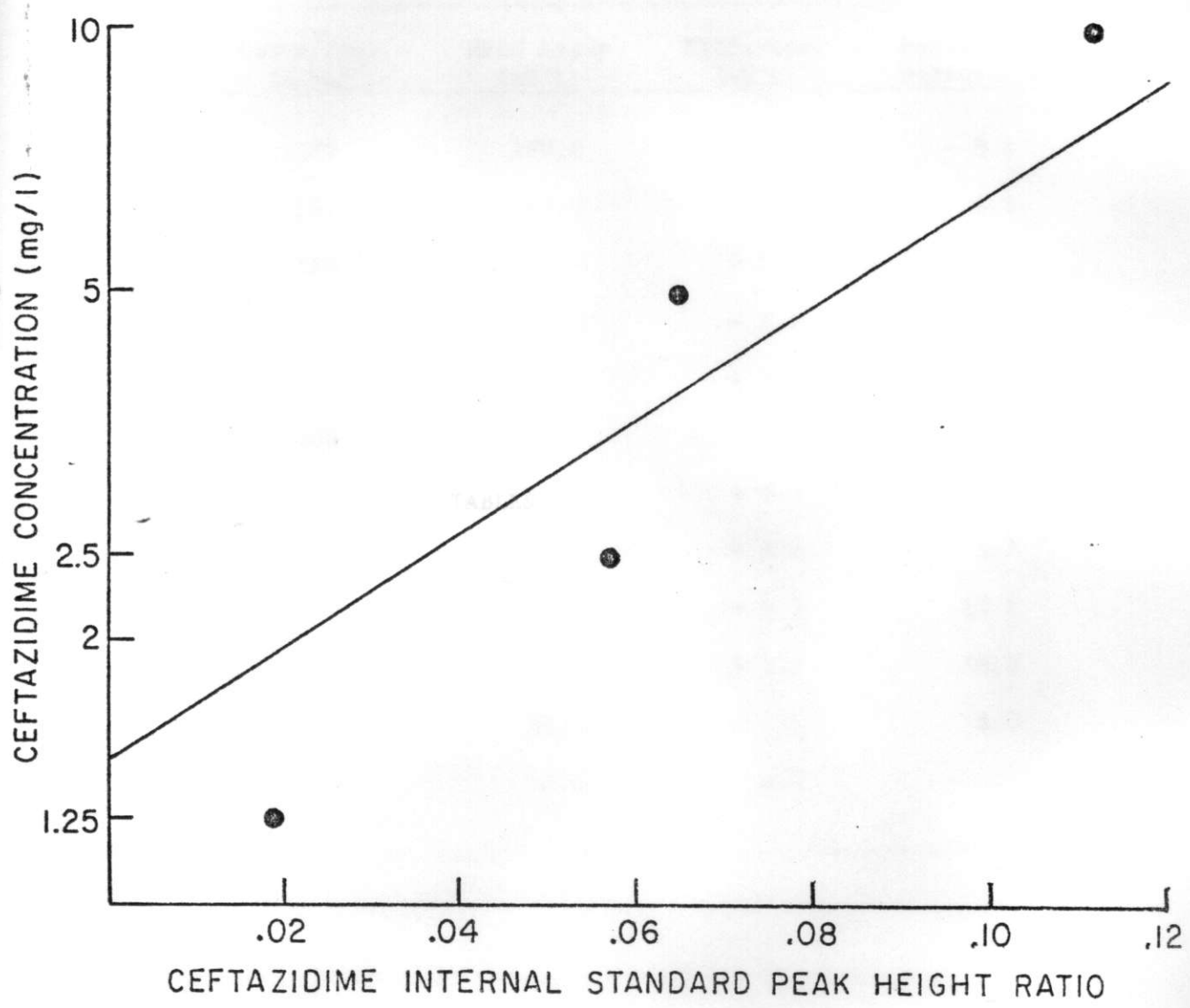

Figure 2. HPLC STANDARD CURVE

TABLES

TABLE 1. Known Concentrations Versus HPLC Assay Results

Sample Number	Known Conc. (mG/L)	HPLC Assay (mG/L)	Difference (mG/L)	Percentage Difference
1	180	180.2	- 0.2	- 0.1
2	167	165.2	+ 1.8	1.1
3	150	140.5	+ 9.5	6.3
4	133	136.2	- 3.2	- 2.4
5	120	116.3	+ 3.7	3.1
6	100	101.0	- 1.0	- 1.0
7	80	71.0	+ 9.0	11.3
8	67	64.5	+ 2.5	3.7
9	50	43.7	+ 6.3	12.6
10	33	29.4	+ 3.6	10.9
11	20	18.4	+ 1.6	8.0
12	10	10.1	- 0.1	- 1.0

TABLE 2. Known Concentrations Versus Microbiologic Assay Results

Sample Number	Known Conc. (mG/L)	Microbiologic Assay (mG/L)	Difference (mG/L)	Percentage Difference
1	180	173	+ 7	3.9
2	167	161	+ 6	3.6
3	150	125	+ 25	16.7
4	133	106	+ 27	20.3
5	120	124	- 4	- 3.3
6	100	92	+ 8	8.0
7	80	65	+ 15	18.8
8	67	57	+ 10	14.9
9	50	46	+ 4	8.0
10	33	29	+ 4	12.1
11	20	*	*	*
12	10	10	0	0.0

*No result due to technical difficulties

REFERENCES

1. Harper PB. The in-vitro properties of ceftazidime. J Antimicrob Chemo 1981; 8:5-13.

2. Phillips I, Warren C, Shannon K et al. Ceftazidime: in vitro antibacterial activity and susceptibility to beta-lactamases compared with that of cefotaxime, moxalactam, and other beta-lactam antibiotics. J Antimicrob Chemo 1981; 8:23-31.

3. Neu HC and Labthavikal P. Antibacterial activity and beta-lactamase stability of ceftazidime, an aminothiazolyl cephalosporin potentially active against Pseudomonas aeruginosa. Antimicrob Ag Chemo 1982; 21:11-18.

4. Sakata Y, Boccazzi A, and McCracken G. Pharmacokinetics and bacteriological effect of ceftazidime in experimental Streptococcus pneumoniae, Haemophilus influenzae, and Escherchia coli meningitis. Antimicrob Ag Chemo 1983; 23:213-217.

5. Thornton JE. The microbiological assay of ceftazidime. J Antimicrob Chemo 1981; 8:225-226.

6. Ayrton J. Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. J Antimicrob Chemo 1981; 8:227-231.

7. Barry AL. The Antimicrobic Susceptibility Test: Principles and Practices. Philadelphia: Lea & Febiger, 1976.

8. Sabath LD and Anhalt JP. Assay of Antimicrobics. In: Lennette EH, Balows A, Hausler WJ, Truant JP, eds. Manual of Clinical Microbiology Third Edition. Washington, DC: American Society of Microbiology 1980, pp. 485-490.

9. Freeman DH. Liquid chromatography in 1982. Science 1982; 218: 235-241.

10. Hartwick RA and Brown RP. The use of high pressure liquid chromatography in clinical chemistry and biomedical research. Adv Clin Chem 1980; 21:25-99.

CURRICULUM VITAE

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PERSONAL DATA

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Date of Birth: February 7, 1958

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EDUCATIONAL BACKGROUND

Doctor of Pharmacy
University of Utah
July 1981 - June 1983

Graduate Gerontology Certificate
University of Utah
September 1981 - May 1983

Bachelor of Pharmacy
Washington State University
September 1976 - June 1981

EDUCATIONAL EXPERIENCE

Residency in Clinical Pharmacy
Department of Pharmacy Practice
University of Utah

Duties: Teaching (clerkships, didactic), clinical rotations, night call, cardiac arrest team participation, clinical seminars, journal club, committee meetings, poison center coverage

EDUCATIONAL EXPERIENCE (continued)

Clinical Rotations

Infectious Disease	12 weeks
Adult Medicine	12 weeks
Geriatrics	12 weeks
Pediatrics	12 weeks
General Surgery	6 weeks
Drug Information	6 weeks
Obstetrics and Gynecology	6 weeks
Psychiatry	6 weeks
Toxicology	6 weeks
Family Practice	6 weeks
Hospital Pharmacy Management	3 weeks

PROFESSIONAL EXPERIENCE

Intermountain Regional Poison Control Center

Salt Lake City, UT
July 1981 - present
Supervisor: Brent Ekins, Pharm.D.

Poison Information Pharmacist (part-time)

Duties: provide telephone toxicology consults; consult on treatment in emergency room cases; teach students during toxicology clerkships

U.S. Public Health Service Hospital

Seattle, WA
Summers, 1979-1980
Supervisor: Alfred Fallavollita, R.Ph.

Pharmacy Intern

Duties: inpatient and outpatient dispensing, IV admixtures, unit dose, nursing unit inspections

TEACHING EXPERIENCE

Teaching Assistant (University of Utah)
Clinical rotations for undergraduate pharmacy students and first year Pharm.D. candidates, 1981-1983

Teaching Assistant (University of Utah)
Common Medicines - course for general university students, 1981-1983

Teaching Assistant (Washington State University)
Basic Pharmaceutics for fourth year pharmacy students, 1980-1981

TEACHING EXPERIENCE (continued)

"Management of Acetaminophen Poisoning" presented for the Advanced Clinical Toxicology course for the first year Doctor of Pharmacy candidates, University of Utah, Spring 1982

"Management of Hypertension" presented for the Advanced Pharmacotherapeutics course to the first year Doctor of Pharmacy candidates, University of Utah, Fall 1982

"Management of Tricyclic Antidepressant Toxicity" presented for the Clinical Toxicology course to the undergraduate pharmacy students, University of Utah, Fall 1982 and Winter 1983

"Headache" presented for the Diseases and Drug Therapy course to the undergraduate pharmacy students, University of Utah, Winter 1983

"Management of Salicylate Poisonings" presented for the Clinical Toxicology course to the undergraduate pharmacy students, University of Utah, Winter 1983

INVITED PRESENTATIONS

"Drug Use in the Elderly" American Association of Retired Persons, Salt Lake City, January 1983

"Therapy of Common Pediatric Infections" Utah Pediatric Nurse Practitioners Association, Salt Lake City, January 1983

"The New Cephalosporins and Penicillins" Medical Residents' Noon Conference, Holy Cross Hospital, Salt Lake City, November 1982

"Beta-blockers" Nursing staff, Holy Cross Hospital, Salt Lake City, October 1982

"Management of Parkinson's Disease" Nursing staff, Geriatric Treatment and Evaluation Unit, Veteran's Administration Medical Center, May 1982

"Use of Digitalis Glycosides" Nursing staff, University of Utah Medical Center, May 1982

"Drug Holidays in Neuroleptic Therapy" Medical staff, Psychiatric Unit, University of Utah Medical Center, November 1981

"Use of Long-Acting Antipsychotics" Medical staff, Psychiatric Unit, University of Utah Medical Center, November 1981

RESEARCH IN PROGRESS

Comparison of 8 Percent Arildone Cream and 5 Percent Acyclovir Ointment in the Topical Treatment of Cutaneous Viral Infections in Immunosuppressed Patients. Co-investigator.

Effect on Serum N-Acetylcysteine Levels Following Therapeutic Doses of Activated Charcoal in Humans. Principal Investigator. (Protocol submitted for funding)

PUBLICATION

Ford D. Moxalactam. Drugs in Patient Care 5(2):6, 1982.

PROFESSIONAL AFFILIATIONS AND ACTIVITIES

American Society of Hospital Pharmacists, 1979-present
Washington State Pharmaceutical Association, 1978-1981
Student American Pharmaceutical Association, 1977-1981
American Association of Colleges of Pharmacy, 1979-1981
Regional Representative to Student Section, 1979-1980

UNIVERSITY SERVICE

Student Advisory Council Member
University of Utah
1982-1983

Pharmacy Students Advisory Council
Vice President
Washington State University
1979-1981

HONORS AND ACHIEVEMENTS

Pharmacy Faculty Award for Outstanding Senior, 1981
Washington State University

Bristol Award (for highest senior grade average), 1981
Washington State University

Merck, Sharp & Dohme Award, 1981
Washington State University

Rho Chi Society, 1980
Washington State University Chapter
Washington State University Outstanding Senior Woman